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Full Length Research Paper

Antitumoral effect of Striga hermonthica (Delile) Benth. methanolic extract is mediated by alterations on procaspase-3 and cyclin B expression in prostate cancer cell lines

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Cancer is one of the major public health problems of the 21st century. Its incidence is increasing in both developed and low-income countries. This trend is justified by the change in people's lifestyles. Cancer of the prostate is one of major cancer types affecting male population. The present study focused on the use of methanolic extract of *Striga hermonthica* (Delile) Benth. to evaluate total phenolics and flavonoids contents, antioxidant activities, anti-proliferative and anti-migratory effects, using ATCC prostate cancer cell lines (22RV1, DU145, C4-2 and RWPE-1). Total phenolics and flavonoids content were estimated using spectrophotometric methods. Antioxidant potential was evaluated using three methods while cytotoxicity assay, anti-migratory assay and western blot analysis were used for the determination of extracts anti-proliferative activity on prostate cancer cells. Therefore, to assess apoptosis induction in prostate cancer cell lines, the effect of *S. hermonthica* (Delile) Benth. methanolic extract was investigated on procaspase-3, procaspase-8, and cyclin-B_2 expression. A range of half-dilution concentrations beginning from 500 µg/mL have been prepared to determine the IC_{50} in the various tests. Antioxidant compounds were shown to be promising biomolecules, with particular interest in cancer management. Among plant derivative compounds, phenolics and derived molecules have endorsed antioxidant capacities and potential anti-proliferative activities. The best inhibition was obtained on C4-2 cells with an IC_{50} = 65.76 µg/mL and a selectivity index of 2.35. The results obtained from this study could partially justify the traditional indications of this plant in the treatment of prostate cancer.

Key words: Plant derived products, anti-tumoral, prostate cancer, antioxidants.

INTRODUCTION

Cancer is a chronic, non-communicable disease characterized by a non-controlled cell proliferation

(Salehi et al., 2019). Its incidence has increased in recent years and trends indicate that the future years will not be much brighter. According to the World Health Organization data in 2019, cancers were among the leading causes of death for people over 75 years in 123 of 183 countries (Jawerth, 2019; WHO, 2020). In these, prostate cancer accounts for 13.5% (1,276,106) men in the world.

Cancer medical treatments strategies include chemotherapy, radiotherapy and surgery. Chemotherapy is the most used in management, control and cancer worsening leading to metastasis. Significant advances were made and many adverse side effects were usually observed while using chemicals for treatments.

Elsewhere, medicinal plants have been used in several traditional medicines around the world and were considered as a source of various bioactive compounds (Bouquet, 1969). Then, compounds derived from plants, such as phenolics, terpenes, and alkaloïds were found to exhibit potential antioxidant capacities and were able to control different cancer cells proliferation (Guo et al., 2017; Wang et al., 2018; Salehi et al., 2019). Then also, authors showed that plant extracts significantly inhibited the growth of prostate cancer cell lines, such as PC-3, DU145, RM-1, and C4-2B at varying concentrations between 31.25 and 250 µg/mL (Guo et al., 2017).

Striga hermonthica (Delile) Benth (Scrophulariaceae), a plant used in traditional medicine in parts of Africa (Atawodi et al., 2003; Kiendrebeogo et al., 2005; Koua et al., 2011) was associated with different treatments such as hepatitis and cancers (Choudhury et al., 1998; Koua et al., 2011). More recently, researchers have demonstrated that strigolactones, terpenic lactone from *S. hermonthica* play a key role in the control of pathways related to apoptosis and inflammation (Dell'Oste et al., 2021). The goal of the present study was to investigate cytotoxicity on prostate cancer cell lines and antioxidant activity against *S. hermonthica* methanolic extract (total phenolics and flavonoids contents). More specifically, we evaluated: (1) the antioxidant activity through three methods, (2) cytotoxicity assay, (3) anti-migratory assay and (4) western blot analysis on 22RV1, DU145, C4-2 and RWPE-1 cells against phytochemical screening.

MATERIALS AND METHODS

Ethnobotanic survey site

Ethnobotanical study was realized in Burkina Faso, specifically in Bobo-Dioulasso, the country's second capital city which is approximately at 360 km away from Ouagadougou, the capital city. The investigation site covers about 136.8 km and is located in the South-Western part of Burkina Faso, at 11°15'049' North latitude, 004°26'08,6' West longitude, and an altitude of 445 m. The region is mainly inhabited by Bobos, Mossi, Dafing, and other ethnic groups such as Samogo, Fulani and Lobi/Dagara. The local language is Dioula.

Data collection methods

The survey was carried out from April to June 2019. Plants specimens were localized in the classified reserve of Dindérésso (30 km southern direction from Bobo Dioulasso) and then collected in August 2019. Plant identification was done by a botanist of the Department of Botany of the University Nazi BONI (Bobo Dioulasso, Burkina Faso).

Plant material sampling and preparation

S. hermonthica plant samples were collected and dried before being ground into powder. A sample of 15 g of powder from each sample was weighed and then loaded into Soxhlet extraction cartridges. A volume of 200 mL of methanol was used for extraction and the temperature was maintained at 70°C at least 4 h for each sample. After that, extract was concentrated and placed in empty Petri dishes previously labeled, weighed and dried outdoors at atmospheric pressure.

Chemical and reagents

All solvents were of analytical grade. Agilent Cary 60 UV-Vis Spectrophotometer (Thermo Fisher; GENESYS 30, USA) was used in all spectrophotometric measurements. Ascorbic acid, ferric chloride, aluminium chloride, potassium acetate, quercetin, DPPH reagent, Folin-Ciocalteau reagent, gallic acid, sodium carbonate, and methanol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Millipore deionized water was used throughout: Thiazolyl Blue Tetrazolium Bromide (Sigma Aldrich, USA) and Dimethyl Sulfoxide (Sigma Aldrich, USA).

Determination of total phenolics content (TPC)

Folin-Ciocalteu method was used to estimate total phenolic content according to Nurmi et al. (1996) method, using a linear regression of gallic acid standard curve at 760 nm in the range from 3.125 to 200 mg/L ($Y = 0.003x+0.016$; $R^2 = 0.997$). 250 µl of extract was mixed with 2.5 ml of deionized water. Afterwards, 125 µL Folin-Ciocalteu reagent was added. After 5 min, 375 µL of 20% Na_2CO_3 was added and incubated for 2 h at room temperature. Absorbance was measured at 760 nm on a Spectroscopic Analysis Mecasys (Optizen). Three replicates of each sample were analyzed and the TPC was expressed as mg of gallic acid equivalent per 100 mg of dry extracts (mg GAE/100 mg).

Determination of flavonoids content (FC)

Flavonoid's content was determined according to Lauranson-Broyer and Lebreton (1993) by linear regression of quercetin standard curve at 425 nm in the range of 100 to 1400 µg/mL ($Y = 0.025x +$

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0.014, R^2 = 0.998). The curve was registered after the addition of 60 µL of a freshly prepared 5% (w/v) aluminium chloride solution to 1 mL of different concentrations of quercetin solution. Absorbance was immediately registered after the addition of aluminium chloride, at 425 nm, using a Spectronic Genesys 2 spectrophotometer (Rochester, New York, USA). This reaction was specific to flavonoids containing orthodihydroxyl groups, able to form complexes between the aluminium, C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavanols; complexes were also formed between the aluminium and the orthodihydroxyl groups in A- or B-ring of flavonoids (Mabry et al., 1970). The flavonoids content in three replicates of each plant sample was also registered after the addition of aluminium chloride and was expressed as mg of quercetin equivalent per 100 mg of dry extract (mg QE/100 mg).

Antioxidant activity using $ABTS^{•+}$ method

The radical scavenging capacity of antioxidants for the $ABTS^{•+}$ (2,2'-azinobis-3- ethylbenzothiazoline-6-sulphonate) radical cation was determined as described by Compaoré et al. (2011), with modifications. For each extract, a methanolic solution (10 mg/mL) was diluted 100-fold in distilled water. 10 µL of the sample (diluted solution) was taken and then mixed with 990 µL of the fresh solution from $ABTS^{•+}$. The mixture was incubated in the dark for 15 min. Three replicates of each sample were analyzed and evaluated using a linear regression of gallic acid standard curve at 734 nm in the range from 3.125 to 200 mg/L (y= -7.874.10-4 x+0.709; R^2 = 0.9993). The $ABTS^{•+}$ radical scavenging capacity was expressed as mg of ascorbic acid equivalent per 100 mg of dry extracts (mg AAE/100 mg).

DPPH radical scavenging activity

The antioxidant activity of the extracts was assessed based on their ability to scavenge free radicals as described previously by Kiendrebeogo et al. (2005). Various concentrations of the methanolic plant extracts were prepared (0.15 to 1.5 mg/mL). A methanolic solution of 1-diphenyl-2-picrylhydrazyl (DPPH: 3.8 mL, 60 µg/mL) was rapidly mixed with the plant extract (200 µl, 30 mg/mL) in a test tube, with methanol serving as the blank sample and control was also assayed simultaneously. The contents of the tubes were swirled and then allowed to stand for 30 min at room temperature in the dark. The absorbance was measured at 517 nm in a spectrophotometer.

The scavenging ability of the plant extract was calculated using this equation:

$$\text{DPPH scavenging activity } (\%) = \left(\frac{Abs.control - Abs.sample}{Abs.control}\right) \times 100$$

where: Abs control is the absorbance of DPPH + Methanol; Abs sample is the absorbance of DPPH radical + Sample (sample or standard).

Three replicates of each sample were analyzed and the DPPH radical scavenging capacity was expressed as mg of ascorbic acid equivalent per 100 mg of dry extracts (mg AAE/100 mg).

Iron (III) reduction activity (FRAP)

The method used is described according to the protocol of Dakio et al. (2020). Stock solutions (10 mg/mL) were diluted to the hundredth in distilled water to give a final test concentration of 100 µg/mL. In 3 test tubes, 0.5 mL of the diluted solution and 0.5 mL of distilled water were added to another tube for the blank. A volume of 1.25 mL phosphate buffer (0.2 M; pH 6.6) and 1.25 mL potassium hexacyanoferrate [K_3Fe $(CN)_6$] were added to these tubes. This was heated in a water bath at 50°C for 30 min. After this operation, a volume of 1.25 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 3000 revolutions per minute for 10 min. 0.625 mL of the supernatant were removed from each tube and added to tubes containing 0.625 mL of distilled water. 125 µL of freshly prepared trichloroferrate [$FeCl_3$ (0.1%)] was added to the resulting mixture. Three replicates of each sample were analyzed using a linear regression of ascorbic acid standard curve at 700 nm (y = 3.270.10-3 x; R^2 = 0.9990). The ferric reduction capacity was expressed as mg of ascorbic acid equivalent per 100 mg of dry extracts (mg AAE/100 mg).

Anti-tumoral assays

Cell culture: All cell lines (22RV1, DU145, C4-2 and RWPE-1) were obtained from American Type Culture Collection (Rockville, MD, USA). Cancer cell lines were grown in RPMI 1640 containing 10% fetal bovine serum (FBS) in the presence of 100 U ml^{-1} of penicillin and 0.1 g l^{-1} of streptomycin. Normal prostate cell lines RWPE-1 were grown in defined keratinocyte serum free medium (DKSFM) containing epidermal growth factor (EGF), insulin and fibroblast growth factor (FGF). Cells were incubated at 37°C with 95% air and 5% CO_2. All cell lines were maintained below passage 20 and used in experiments during the linear phase of growth.

Cytotoxicity assay: This was performed as described by Feoktistova et al. (2016). Cells (10^4 per well for 96-well plates) or (10^5 per well for 24-well plates) were seeded for 24 h and treated with the different concentrations (3.9 to 500 µg/mL). After incubation time, cells were washed with water and stained with crystal violet solution. After drying, the crystal violet was dissolved in methanol and absorbances were recorded at 595 nm, the images were recorded with a Wallac Victor 2 (Perkin Elmer) plate reader. Cytotoxicity curves were constructed to estimate the IC_{50} and confidence intervals. Experiments were performed in triplicate and IC_{50} was expressed in µg/mL. Index of selectivity was calculated comparing cytotoxicity on cancer cell lines and normal prostate cells.

Anti-migratory assay: Cancer cells' mobility was evaluated by wound-healing assay (Luanpitpong et al., 2010). Briefly, a monolayer of cells at a density of 1.5 × 10^4 cells per well was cultured in 96-well plates, and a wound space was created by a 1 mm width tip. After rinsing with PBS, the cell monolayers were treated with non-toxic concentration of compounds (0.1 µg/mL) and allowed to migrate for 0 to 48 h. Micrographs were taken under a phase-contrast microscope (×100; Olympus IX51 with DP70), and wound spaces were measured from 10 random fields of view using Olympus DP Controller Software. Quantitative analysis of cell migration was performed by using an average wound space from random fields of view.

Western blot analysis: Prostate cancer cell lines (DU145, 22RV1 and C4-2) were treated with the extraction for 48 h. Thereafter, western blot analysis of procaspase 3, procaspase 8 and cyclin B2 expressions were performed using 50 µg of cell lysates according to the method of Martínez-Flores et al. (2017) with modifications. Results were represented by blots and indicated by densitometric values. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control and experiments were performed in triplicate.

Table 1. Phytochemical content and antioxidant activities of *S. hermonthica* methanol extract.

Extract	Phytochemical content		Antioxidant activity		
	Total polyphenol (mg GAE/100 mg)	Total flavonoid (mg QE/100 mg)	FRAP (µmol AAE)	$ABTS^{\bullet+}$ (µmol AAE)	$DPPH^{\bullet}$ (µmol AAE)
EmeOH	10.78 ± 0.12	8.65 ± 0.13	6.11 ± 0.00	160.86 ± 1.93	3.65 ± 0.17
Trolox	-	-	2211.24±36.17	8137.61±229.5	765.99±17.70
Quercetin	-	-	5991.29±75.56	14474.73±213.43	645.58±3.20

EmeOH: Methanol extract of *S. hermonthica*; GAE: gallic acid equivalent; QE: quercetin equivalent; AAE: ascorbic acid equivalent.

Table 2. IC_{50} (µg/mL) and 95% confidence intervals of *S. hermonthica* methanol extract on normal and prostate cancer cell lines.

Cell line	IC_{50} (µg/mL)	95% Confidence Intervals	SI
DU145	101.9	77.60 - 133.9	1.52
22RV1	81.68	63.04 - 105.8	1.89
C4-2	65.76	47.47 - 91.10	2.35
RWPE-1	154.5	126.7 - 188.4	-

SI: Selectivity index.

Statistical analysis

Phytochemistry and antioxidant activity

All the reactions were performed in triplicate, and data were presented as mean values ± standard deviation (SD). Data were analyzed by one-way analysis of variance followed by Tukey multiple comparison test. Analysis was done using XLSTAT7.1 software. A *p-value* < 0.05 was considered as a criterion for statistical significance.

Cytotoxicity

Results were presented as mean values ± SD. Data were analyzed by either one-way ANOVA followed by Dunnet's test $\alpha = 0.05$ to compare experimental means with controls using GraphPad Prism 4 software. To compare statistical differences between theoretical and experimental isobolograms, Student's t-test was performed and $p \leq 0.05$ was significant (Lynn et al., 2006).

RESULTS AND DISCUSSION

Several scientific authors have admitted that using natural resources is an important element of public health in many African countries for historical, cultural and social reasons (Bouquet, 1969; Bangou et al., 2012). According to the WHO (2002), modern and traditional medicines were based on medicinal plants which constitute an effective source of bioactive compounds.

The evaluation of total phenolics and flavonoids content of methanolic extract of *S. hermonthica* (Delile) Benth and their antioxidant ability to reduce ferric ion and scavenging $ABTS^{\bullet+}$ and DPPH radical was shown in Table 1. The total phenolic of methanolic *S. hermonthica* extract was estimated to 10.78 mg GAE/100 mg of dry extract, including ortho-hydroxyl-flavonoids estimated at 8.5 mg QE/100 mg of dry extract. The antioxidant screening of the methanolic extract of *S. hermonthica* showed a weak activity when compared with trolox and quercetin. The $ABTS^{\bullet+}$ radical scavenging capacity showed the best antioxidant activity (160.86 ± 1.93 µmol AAE), followed respectively by ferric ion reduction ability (6.11 ± 0.00 µmol AAE) and DPPH radical scavenging (3.65 ± 0.17 µmol AAE). These results were in coherence with those of Kiendrebeogo et al. (2005) which showed that *S. hermonthica* extracts containing polyphenolic compounds include orthohydroxyl-flavonoids that exhibit high antioxidant activity.

The anti-proliferative effect of *S. hermonthica* methanolic extracts on prostate cell lines indicated that tumor cell lines (DU145, 22RV1 and C4-2) were most sensitive with IC_{50} from 101.9 to 65.76 µg/mL compared to normal cell line (RWPE-1) with IC_{50} at 154.5 µg/mL (Table 2 and Figure 1). Thereafter, C4-2 cell line exhibited more selectivity index (SI = 2.35) compared to normal prostate cell line RWPE-1 (Table 2).

Cytotoxicity activities of plants extracts were described in previous investigations on prostate cancer and/or urinary tract related-diseases (Turan et al., 2015; Foster et al., 2020). Some plant species showed anti-proliferative activities against various prostate cancer cell lines (DU-145, PC-3, and LNCaP, Pca and hTert-PrEC) (Shabbeer et al., 2009; Petiwala et al., 2013; Foster et al., 2020), respectively, *Trigonella foenum-graecum* (IC_{50} = 100 µg/mL on MDA-MB-231), *Rosmarinus officinalis* (IC_{50} = 41.1 µg/mL on PC-3) and *Annona muricata* L (IC_{50} = 0.1 µg/mL on DU145 and 55 µg/mL on DU145) using MTT

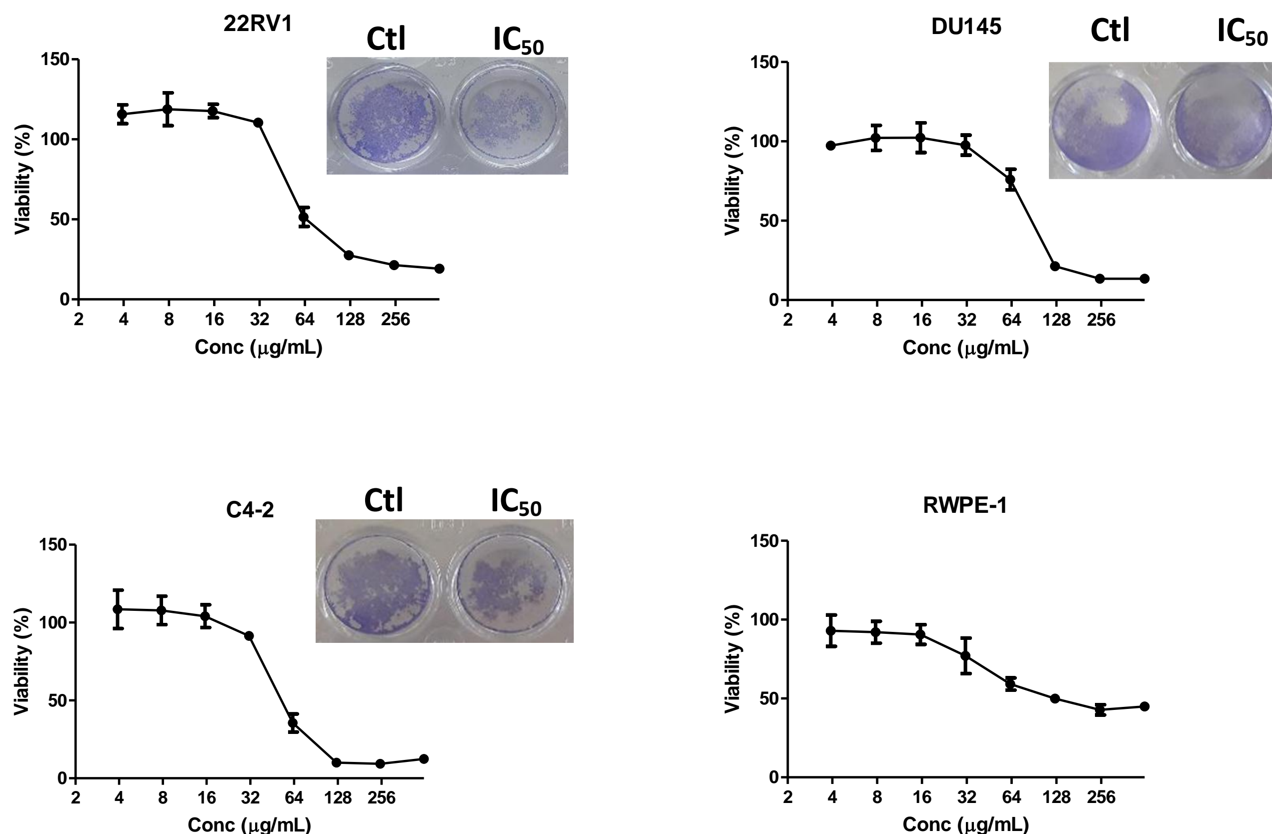

Figure 1. Cytotoxicity effect of *S. hermonthica* methanol extract on prostate cancer and normal cell lines.

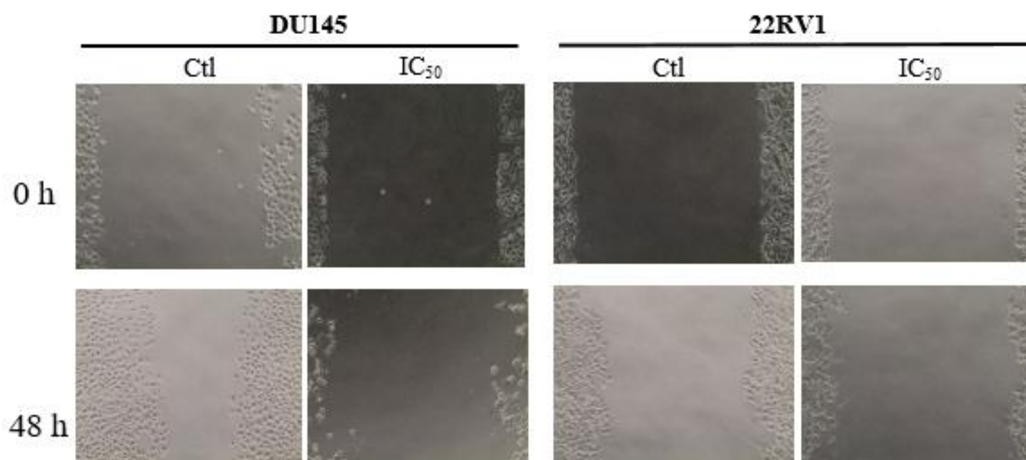

Figure 2. Methanol extract of *S. hermonthica* inhibits the migration of prostate cancer cell lines.

assay. Plant-derived compounds were responsive to plants bioactivities. Curcumim from *Curcuma longa* L. species exhibited an IC_{50} of between 0.68 and 0.78 µg/mL on prostate cancer cell lines (Chen et al., 2018). The effect of *S. hermonthica* methanolic extracts on cell migration was evaluated, using prostate cancer cell lines DU145 and 22RV1 in order to assess the extract's ability to inhibit metastases dissemination (Table 2). These results showed, anti-migratory effect on DU145 prostate cancer cell lines more than 22RV1 cell lines after 48 h treatment (Figure 2). Our observed results after 48 h treatment were according to the literature that

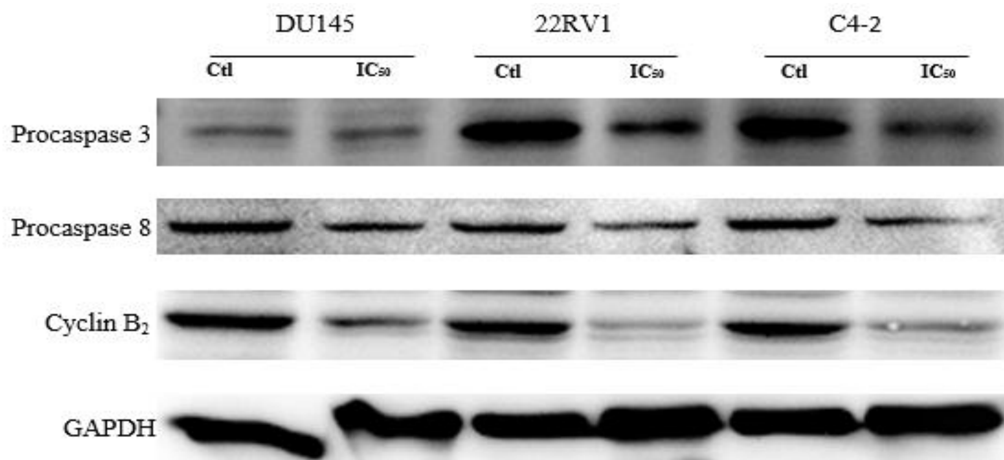

Figure 3. Alteration of procaspase 3 and 8, and cyclin B_2 proteins levels from prostate cancer cells incubated with *S. hermonthica* methanol extract (µg/mL).

attributed response delay to the repair of DNA induced by plant extracts (Lindahl, 2000; Boer and Hoeijmakers, 2000).

Apoptosis occurs when cellular DNA is damaged and is induced by caspases activating cascade. Active caspases, cysteine proteases that initiate and execute the apoptotic program, are formed by proteolyze of procaspases. Tumor cells exhibited a resistance to apoptosis associated with the overexpression of procaspases. Thereafter, the reactivation of apoptotic cascades was a strategic approach to new anti-cancer drug discovery (Lamas et al., 1993; Saleli et al., 2019; Foster et al., 2020; Dell'Oste et al., 2021). The conversion of procaspase-3 to caspase-3 is a crucial node of apoptosis and is often considered as "the end executioner caspase of no return" for a cell. The overexpression of procapase-3 in tumor cell has been reported in various cancer types. Herein, procapase-3 activators can be considered as good targets in anticancer strategy (Vegran et al., 2005; Couzinet et al., 2002). According to the literature, cyclins would be a good marker for cells, particularly tumour cells (Lamas et al., 1993). Cyclin B_2 is a mitotic cyclin that inappropriate overexpression causes non-specific cell death in absence of mitotic arrest (Vegran et al., 2005). We investigated the alteration of procaspase-3, procaspase-8 and cyclin B_2 expression, in DU145, 22RV1 and C4-2 prostate cancer cell lines, incubated 48 h at IC_{50} (using different concentrations: 0, 3.9, 7.81, 15.625, 31.25, 62.5, 125, 250 and 500 µg/mL). Observed blots (Figure 3) showed that procaspase-3 expression was more potent in 22RV1 (IC_{50} = 81.68 µg/mL) and C4-2 (IC_{50} = 65.76 µg/mL) cell lines compared to DU145 (IC_{50} = 101.9 µg/mL). *S. hermonthica* methanol extract induced a reduction in procaspase-3 and cyclin B_2 content in 22RV1 and C4-2 cell lines, compared to DU145 cancer cell line where low alteration of these proteins expression were shown. Alterations in procaspase-8 expression were similar in all tested prostate cancer cell lines. These results suggested that the anti-tumoral activity of *S. hermonthica* methanol extract on prostate cancer cell lines 22RV1 and C4-2 could be related to caspase-3 activation leading to antiproliferative effect. Several studies have reported that polyphenolic compounds such as flavonoids and anthocyanins with their glycosides induce death of many different cancer cell lines linked to caspase-dependent apoptosis (Couzinet et al., 2002; Gasmi, 2012; Guo et al., 2017; Sagbo and Otang-Mbeng, 2021; Dell'Oste et al., 2021).

Variations in the procaspases expression have been linked to tumour progression and chemoresistance (Vegran et al., 2005). Thus, the inhibition of these tumor-related proteins was a major objective in the research of active biomolecules against cancer.

Conclusion

In this study, anti-tumoral effect of *S. hermonthica* methanolic extract is mediated by alterations on procaspase-3 and cyclin B2 expression in prostate cancer cell lines. This extract also exhibited a correlation between total phenolics and flavonoids content with antioxidant activity. In addition, eight increasing half-dilution concentrations ranging from 3.9 to 500 µg/mL were prepared to determine the corresponding inhibitory concentrations of 50% cancer cells. All the cells (22RV1, DU145 and C4-2) used showed inhibition levels of below 125 µg/mL. The best inhibition was obtained on C4-2 cells with an IC_{50} of 65.76 ml and a selectivity index of 2.35. Thus, the findings suggest that the cytotoxic effect may be due to apoptosis induction through caspase dependent pathways. The data showed *S. hermonthica* methanolic extract as a potential source of anti-tumoral

compounds, activating an apoptotic cascade in cancer cells. Indeed, interest in apoptotic proteins as anti-cancer targets can be considered in the discovery of several promising new drugs. In addition, the identification of active compounds targeting procaspase-3 expression could lead to the development of novel anti-tumoral drugs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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